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METHODS AND COMPOSITIONS FOR TREATING HEPATITIS C VIRUS

FIELD OF THE INVENTION

This invention is in the area of pharmaceutical chemistry, and in particular, is a compound, method and composition for the treatment of hepatitis C virus. This application is a continuation of U.S. application Ser. No. 09/864,078, filed on May 23, 2001, now U.S. Pat. No. 6,914,054, which claims priority to U.S. provisional application No. 60/206,585, filed on May 23, 2000, the disclosures of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The hepatitis C virus (HCV) is the leading cause of chronic liver disease worldwide. (Boyer, N. et al. *J. Hepatol.* 32:98-112, 2000). HCV causes a slow growing viral infection and is the major cause of cirrhosis and hepatocellular carcinoma (Di Besceglie, A. M. and Bacon, B. R., *Scientific American*, Oct.: 80-85, (1999); Boyer, N. et al. *J. Hepatol.* 32:98-112, 2000). An estimated 170 million persons are infected with HCV worldwide. (Boyer, N. et al. *J. Hepatol.* 32:98-112, 2000). Cirrhosis caused by chronic hepatitis C infection accounts for 8,000-12,000 deaths per year in the United States, and HCV infection is the leading indication for liver transplant.

HCV is known to cause at least 80% of posttransfusion hepatitis and a substantial proportion of sporadic acute hepatitis. Preliminary evidence also implicates HCV in many cases of "idiopathic" chronic hepatitis, "cryptogenic" cirrhosis, and probably hepatocellular carcinoma unrelated to other hepatitis viruses, such as Hepatitis B Virus (HBV). A small proportion of healthy persons appear to be chronic HCV carriers, varying with geography and other epidemiological factors. The numbers may substantially exceed those for HBV, though information is still preliminary; how many of these persons have subclinical chronic liver disease is unclear. (The Merck Manual, ch. 69, p. 901, 16th ed., (1992)).

HCV has been classified as a member of the virus family Flaviviridae that includes the genera flaviviruses, pestiviruses, and haptociviruses which includes hepatitis C viruses (Rice, C. M., Flaviviridae: The viruses and their replication. In: Fields Virology, Editors: Fields, B. N., Knipe, D. M., and Howley, P. M., Lippincott-Raven Publishers, Philadelphia, Pa., Chapter 30, 931-959, 1996). HCV is an enveloped virus containing a positive-sense single-stranded RNA genome of approximately 9.4 kb. The viral genome consists of a 5' untranslated region (UTR), a long open reading frame encoding a polyprotein precursor of approximately 3011 amino acids, and a short 3' UTR. The 5' UTR is the most highly conserved part of the HCV genome and is important for the initiation and control of polyprotein translation. Translation of the HCV genome is initiated by a cap-independent mechanism known as internal ribosome entry. This mechanism involves the binding of ribosomes to an RNA sequence known as the internal ribosome entry site (IRES). An RNA pseudoknot structure has recently been determined to be an essential structural element of the HCV IRES. Viral structural proteins include a nucleocapsid core protein (C) and two envelope glycoproteins, E1 and E2. HCV also encodes two proteinases, a zinc-dependent metalloproteinase encoded by the NS2-NS3 region and a serine proteinase encoded in the NS3 region. These proteinases are required for cleavage of specific regions of the precursor polyprotein into mature peptides. The carboxyl half of nonstructural protein 5, NS5B, contains the RNA-dependent RNA polymerase. The function

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of the remaining nonstructural proteins, NS4A and NS4B, and that of NS5A (the amino-terminal half of nonstructural protein 5) remain unknown.

A significant focus of current antiviral research is directed toward the development of improved methods of treatment of chronic HCV infections in humans (Di Besceglie, A. M. and Bacon, B. R., *Scientific American*, Oct.: 80-85, (1999)). Currently, there are two primary antiviral compounds, Ribavirin and interferon-alpha, which are used for the treatment of chronic HCV infections in humans.

Treatment of HCV Infection with Ribavirin

Ribavirin (1- β -D-ribofuranosyl-1-1,2,4-triazole-3-carboxamide) is a synthetic, non-interferon-inducing, broad spectrum antiviral nucleoside analog sold under the trade name, Virazole (The Merck Index, 11th edition, Editor: Budavari, S., Merck & Co., Inc., Rahway, N.J., p1304, 1989). U.S. Pat. No. 3,798,209 and RE29,835 disclose and claim Ribavirin. Ribavirin is structurally similar to guanosine, and has in vitro activity against several DNA and RNA viruses including Flaviviridae (Gary L. Davis. *Gastroenterology* 118:S104-S114, 2000).

Ribavirin reduces serum amino transferase levels to normal in 40% of patients, but it does not lower serum levels of HCV-RNA (Gary L. Davis. *Gastroenterology* 118:S104-S114, 2000). Thus, Ribavirin alone is not effective in reducing viral RNA levels. Additionally, Ribavirin has significant toxicity and is known to induce anemia.

Treatment of HCV Infection with Interferon

Interferons (IFNs) are compounds that have been commercially available for the treatment of chronic hepatitis for nearly a decade. IFNs are glycoproteins produced by immune cells in response to viral infection. IFNs inhibit viral replication of many viruses, including HCV, and when used as the sole treatment for hepatitis C infection, IFN suppresses serum HCV-RNA to undetectable levels. Additionally, IFN normalizes serum amino transferase levels. Unfortunately, the effects of IFN are temporary and a sustained response occurs in only 8%-9% of patients chronically infected with HCV (Gary L. Davis. *Gastroenterology* 118:S104-S114, 2000).

A number of patents disclose HCV treatments using interferon-based therapies. For example, U.S. Pat. No. 5,980,884 to Blatt et al. discloses methods for retreatment of patients afflicted with HCV using consensus interferon. U.S. Pat. No. 5,942,223 to Bazer et al. discloses an anti-HCV therapy using ovine or bovine interferon-tau. U.S. Pat. No. 5,928,636 to Alber et al. discloses the combination therapy of interleukin-12 and interferon alpha for the treatment of infectious diseases including HCV. U.S. Pat. No. 5,908,621 to Glue et al. discloses the use of polyethylene glycol modified interferon for the treatment of HCV. U.S. Pat. No. 5,849,696 to Chretien et al. discloses the use of thymosins, alone or in combination with interferon, for treating HCV. U.S. Pat. No. 5,830,455 to Valtuena et al. discloses a combination HCV therapy employing interferon and a free radical scavenger. U.S. Pat. No. 5,738,845 to Imakawa discloses the use of human interferon tau proteins for treating HCV. Other interferon-based treatments for HCV are disclosed in U.S. Pat. No. 5,676,942 to Testa et al., U.S. Pat. No. 5,372,808 to Blatt et al., and U.S. Pat. No. 5,849,696.

Combination of Interferon and Ribavirin

The combination of IFN and Ribavirin for the treatment of HCV infection has been reported to be effective in the treatment of IFN naive patients (Battaglia, A. M. et al., *Ann. Pharmacother.* 34:487-494, 2000). Results are promising for this combination treatment both before hepatitis develops or